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(54) Title: CONTROL OF GENE EXPRESSION BY IONIZING RADIATION

(57) Abstract

This invention relates to genetic constructs which comprise an enhancer-promoter region which is responsive to radiation, and at least one structural gene whose expression is controlled by the enhancer-promoter. This invention also relates to methods of destroying, altering, or inactivating cells in target tissue by delivering the genetic constructs to the cells of the tissues and inducing expression of the structural gene or genes in the construct by exposing the tissues to ionizing radiation. This invention is useful for treating patients with cancer, clotting disorders, myocardial infarction, and other diseases for which target tissues can be identified and for which gene expression of the construct within the target tissues can alleviate the disease or disorder.

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CONTROL OF GENE EXPRESSION BY IONIZING RADIATION

This invention relates to methods of controlling gene expression by radiation responsive genetic constructs. This invention also relates to methods and compositions for destroying, altering, or inactivating target tissues. These tissues may be disease-related, for example, tumors, or blood clots, or they may have a metabolic deficiency or abnormality. An aspect of this invention is to deliver radiation responsive genetic constructs to target tissues and to activate the genes in said constructs by exposing the tissues to external ionizing radiation.

Certain genes may play a role in the cellular response to stress or DNA-damaging agents. For example, metallothionein I and II, collagenase, and plasminogen activator are induced after UV irradiation (Angel, et al., 1986; 1987; Fornace, et al., 1988a and b; Miskin, et al., 1981). B2 polymerase III transcripts are increased following treatment by heat shock (Fornace, et al., 1986; 1989a). Furthermore, although the level of DNA polymerase β mRNA is increased after treatment with DNA-damaging agents, this transcript is unchanged following irradiation, suggesting that specific DNA-damaging agents differentially regulate gene expression (Fornace, et al., 1989b). Protooncogene c-*fos* RNA levels are elevated following treatment by UV, heat shock, or chemical carcinogens (Andrews, et al., 1987; Hollander, et al., 1989a). In this regard, the relative rates of *fos* transcription during heat sh ck are unchanged, suggesting that this stress increas d c-*fos* RNA thr ugh posttranscriptional m chanisms (Hollander, et al., 1989b).

Investigations of the cytotoxic effects of ionizing

radiation has focused on the repair of DNA damage or the modification of radiation lethality by hypoxia (Banura, et al., 1976; Moulder, et al., 1984). In prokaryotes and lower eukaryotes, ionizing radiation has been shown to induce expression of several DNA repair genes (Little, et al., 1982); however, induction of gene expression by ionizing radiation has not been described in mammalian cells. DNA-damaging agents other than x-rays induce expression of a variety of genes in higher eukaryotes (Fornace, et al., 1988, 1989; Miskin, et al., 1981).

What is known about the effects of ionizing radiation is that DNA damage and cell killing result. In many examples, the effects are proportional to the dose rate. Ionizing radiation has been postulated to induce multiple biological effects by direct interaction with DNA or through the formation of free radical species leading to DNA damage (Hall, 1988). These effects include gene mutations, malignant transformation, and cell killing. Although ionizing radiation has been demonstrated to induce expression of certain DNA repair genes in some prokaryotic and lower eukaryotic cells, little is known about the effects of ionizing radiation on the regulation of mammalian gene expression (Borek, 1985). Several studies have described changes in the pattern of protein synthesis observed after irradiation of mammalian cells. For example, ionizing radiation treatment of human malignant melanoma cells is associated with induction of several unidentified proteins (Boothman, et al., 1989). Synthesis of cyclin and c regulated polypeptides is suppressed by ionizing radiati n in rat REF52 c lls but not in oncogene-transformed REF52 cell lines (Lambert and Borek, 1988). Other studies have dem nstrated that certain growth factors or cytokines may be involved in x-ray-induced DNA damage. In this regard, platelet-derived growth factor

is released from endothelial cells after irradiation (Witte, et al., 1989).

Initiation of mRNA synthesis by DNA is a critical control point in the regulation of cellular processes and depends on bindings of certain transcriptional regulatory factors to specific DNA sequences. However, little is known about the regulation of transcriptional control by ionizing radiation exposure in eukaryotic cells. The effects of ionizing radiation on posttranscriptional regulation of mammalian gene expression are also unknown.

Many diseases, conditions, and metabolic deficiencies would benefit from destruction, alteration, or inactivation of affected cells, or by replacement of a missing or abnormal gene product. In certain situations, the affected cells are focused in a recognizable tissue. Current methods of therapy which attempt to seek and destroy those tissues, or to deliver necessary gene products to them, have serious limitations. For some diseases, e.g., cancer, ionizing radiation is useful as a therapy. Methods to enhance the radition, thereby reducing the necessary dose, would greatly benefit cancer patients. Therefore, methods and compositions were sought to enhance radiation effects by investigating effects of radiation on gene expression. A goal was to provide new types of therapy using radiation, and to explore other uses of radiation.

In this invention, control exerted over gene expression by a prom ter-enhancer region, which is r sp nsive to ionizing radiati n, is used as a switch to selectively introduc gen pr ducts to distinct tissu targets, providing opp rtunities for therapeutic d struction, alteration, or inactivation of cells in targ t tissues. These promoter-enhancer regions control

gene expression through application of a radiation trigger.

More particularly, this invention relates to methods and compositions for treating diseases and conditions for which destruction, alteration or inactivation of cells in affected tissues would alleviate the disease or condition. The methods comprise delivering a genetic construct to cells of the host tissue and subsequently exposing the tissue to ionizing radiation. A region of the genetic construct is capable of being induced by ionizing radiation. Exposing the tissue to ionizing radiation, therefore, induces the expression of the genetic construct. The gene product is then capable of destroying, altering, or inactivating the cells in the tissue. The gene product chosen for treatment of factor deficiencies or abnormalities, is one that provides the normal n factor.

An illustrative embodiment of the genetic construct comprises a combination of a radiation responsive enhancer-promoter region and a region comprising at least one structural gene. The enhancer-promoter region drives the expression of a structural gene in the form of a reporter-effector gene appropriate for the disease or condition in the host.

The general composition of the construct comprises a radiation inducible promoter-enhancer region and a structural gene region. In an illustrative embodiment, the promoter is 5' to th structural gene region. In this embodiment, amplification of the final respons does not ccur. Rather there is a dir ct c rrelation between regulati n of th radiation sensitive r gion and th structural gene. The inducible region is turned on by radiation exp sur , but will turn ff at some point after

the radiation xposure ceases. Expr ssion f th structural gene region is limited by exposure time and the inherent quantitative limits of the expression region.

In a preferred embodiment, to amplify the expression of the gene construct and to extend expression beyond exposure time, a cascade of promoters and expressing genes are contemplated, for example, two plasmids. Th first plasmid comprises the radiation sensitive promot r 5′ of an appropriate transcription factor. In an embodiment of a transcription factor, the first plasmid comprises a powerful activation domain, for example, that obtained from the herpes virus VP16. This domain contains many negatively charged residues. A chimeric protein is contemplated in this embodiment comprising the VP16 activation domain and a DNA binding domain of a known protein, for example, the lac repressor. The chimeric protein/gene construct (a fusion gene) is driven from a radiation sensitive promoter.

The second plasmid construct in the preferred embodiment comprises several binding sites for the lac repressor DNA binding domain. These binding sites are placed upstream of a reporter-effector gene, for exampl , TNF. Alternatively, the two plasmids described above could be merged into one construct.

The use of a cascade of promoters and two expressing genes as the genetic construct has several advantages:

(1) th promoter d es n t hav to provide strong activati n becaus amplification f the initial radiation sensitiv promoter ffect is provid d through action of th subsequent genetic cascad ;

(2) several g nes may b included in the construct

to provide more complex or m re xtensive action. In an illustrative embodiment, several toxin producing genes may be placed 3′ of the appropriate DNA binding sites. An embodiment of a multiple gene construct comprises the DNA binding domain of the lac repressor followed by several genes which produce various regulators of cell growth; and

(3) the effect due to the initial ionizing radiation may be temporarily prolonged; that is, if the half-life of the chimeric lac repressor protein were long, for example, hours or day, compared to the radiation exposure time during which promoter RNA is released, the effect of the genetic construct on the cell is prolonged.

The genetic construct of this invention is incorporated into the cells of a target tissue by any method which incorporates the construct without inhibiting its desired expression and control over that expression by radiation. These methods comprise electroporation, lipofection, or retroviral methodology.

Retroviruses used to deliver the constructs to the host target tissues generally are viruses in which the 3′ LTR (linear transfer region) has been inactivated. That is, these are enhancerless 3′LTR′s, often referred to as SIN (self-inactivating viruses) because after productiv infection into the host cell, the 3′LTR is transferred to th 5′ end and b th viral LTR′s are inactive with respect to transcriptional activity. A use of these virus s well known to those skilled in the art is to cl ne genes for which the regulatory elements of th cloned gen ar inserted in th space b tw n the tw LTR′s. An advantage of a viral inf ction system is that it allows

for a very high level of infection into th appropriate recipient cell, e.g., LAK cells.

For purposes of this invention, a radiation responsive enhancer-promoter which is 5′ of the appropriate structural gene region, for example, a lymphokyne gene, or a transcriptional activator, may be cloned into the virus.

The constructs are delivered into a host by any method that causes the constructs to reach the cells of the target tissue, while preserving the characteristics of the construct used in this invention. These methods comprise delivering the construct by intravenous injection, injection directly into a target tissue, or incorporation into cells which have been removed from th host. In the latter case, after *in vitro* incorporation of the constructs into the recipient cells, the cells containing the construct are reintroduced into the host. Depending on the type of recipient cell, the distribution of the cells in the host will vary - in some cases being focused to a specific area, for example, where cells are directed to a tumor or clot, in other cases diffusing through an entire system such as the bone marrow. Even when the cells carrying the genetic construct have dispersed over a wide area of the host, focusing the desired action of the construct on a target tissue can be provided by directing the ionizing radiation used to switch on the construct, to a limited area. Only the cells within the beam will react and cause expression of the construct genes.

Another method f f cusing th gen tic action of the construct, or homing it into particular body r gi ns, is to tag the construct with a radioisotope or other label and determine when the c nstruct bearing cells have

reached the target tissue by detecting the label geographically. The radiation is turned on when the construct reaches the target, and directed to the labelled direction.

The type of recipient cells used to incorporate the radiation inducible genetic constructs are selected bas d on the objective of the treatment. In an exemplary embodiment, LAK cells are used for patients in which tumor-directed attack is the main objective. In another embodiment, endothelial cells are used to deliver genes for gene therapy, for example, to treat genetically abnormal fetuses with a metabolic deficiency or abnormality. Cells derived from peripheral blood are also suitable recipient cells.

In an exemplary embodiment of the genetic construct, there are several steps leading to expression of the structural gene in the host tissues. In these constructs, there is a radiation sensitive promoter which causes (drives) the expression of a transcription factor. The transcription factor activates a reporter construct which includes an effector appropriate for the disease r condition of the host. The expression production of the effector gene interacts in a therapeutic fashion with the diseased, deficient or abnormal cells without a target tissue.

In an exemplary embodiment, toxins which are capable of killing tumor cells are put into LAK cells or other cellular/molecular vehicles by incorp rating into the cells a vector comprising a radiation inducible or resp nsive prom ter-enhancer region and a structural gen regi n. Exampl s f a radiation responsive prom ter- nhancer region c mprise that d rived from, for xample, c-*jun* or TNF-α. Examples of structural gen s comprise

th se xpr ssed as tumor n crosis factor (TNF), ricin, r various growth factors including, but not limited to, IL-1-6, PDGF (platelet derived growth factor) or FGF (fibroblast growth factor). Diseases for which this embodiment of a construct is useful comprise cancers. Types of cancers which would benefit from this form of treatment comprise solid and hematologic malignancies. Specific cancers include head and neck adenocarcinomas.

An embodiment of genetic construct comprises a radiation sensitive promoter coupled to an appropriate reporter, for example, β-galactosidase. The construct is transferred to a recipient cell. In general, many recipient cells are prepared in this fashion. The recipient cells are then introduced into a mammal. In an illustrative example, endothelial cells are used as the recipient cells. These cells are then transplanted into an appropriate blood vessel in which the action of the construct within the cells is desired. Radiation is delivered to an area of the body including that blood vessel. Expression of the β-galactosidase is monitored by chromogenic assays such as Xgal.

An embodiment of a structural gene which acts as a reporter-effector gene comprises that which is expressed as the tumor necrosis factor (TNF). Increased TNF-α production by human sarcomas after x-irradiation is evidence for the direct cytotoxic effects of this polypeptide on human tumor cells (Sugarman, 1985; Old, 1985). The intracellular production of TNF-α within irradiated tumor cells results in lethality to the cell after x-ray exposure that is greater than the lethality produced by the direct effects f ionizing radiation alone.

The additiv and synergistic eff cts, th latter

occurring if TNF is provid d b fore radiation, of TNF-α on tumor killing by radiation supports potential applications for the use of TNF-α in clinical radiotherapy. TNF-α potentiates the cellular immune response (Bevelacqua, et al., 1989; Sersa, et al., 1988). *In vivo* studies have shown that TNF-α enhances tumor control by x-rays in mice with implanted syngeneic tumors by the augmentation of the host's immune system (Sersa, et al., 1988). Therefore, TNF-α may reverse immune suppression, which often accompanies radiotherapy. TNF-α also causes proliferation of fibroblasts and endothelial destruction, suggesting that TNF-α production by tumors may be one component responsible for the late radiation effects in surrounding normal tissue. Turning on this gene within a genetic construct by radiation allows directed attack on diseased tissues.

In addition to killing tumor cells by treatment with TNF, a goal is to protect normal tissues adjacent to the target tissue from radiation effects and deleterious action of various cytotoxins during cancer or other therapy. Solid and hemologic malignancies and aplastic anemia, are conditions for which this is a concern. Genes in the structural region of the genetic construct of this invention that are appropriate for this protective goal, include lymphokines, GCSF, CMSF, and erythropoietin.

The goal of cancer treatment is not only to kill cells at a specific target, but to inhibit metastasis. F r this purpose, one of the genes appropriate for inclusion in th genetic construct is NM23.

Prevention of s condary malignancies which are and unf rtunate side effect of standard radiotherapy and chemotherapy, is assisted by treatment with a construct

comprising tum r suppr ssor g nes.

This invention has uses in diseases and conditions other than cancer. For patients with clotting disord rs, Factor VIII or other factors necessary for the complex process of clot formation, may be introduced into cells deficient for the missing factor.

Conversely, in conditions such as myocardial infarction, central nervous system or peripheral thrombosis, anticlotting factors introduced via the genetic constructs of this invention, are used to dissolve the clots. Embodiments of the expression products of such genes include streptokinase and urokinase.

Other categories of diseases or conditions for which there is a deficiency due to either a genetic or environmental factor, include the hemoglobinopathies such as sickle cell anemia, for which genes producing normal hemoglobin are included in the treatment construct; neurodegenerative diseases such as Alzheimer's disease for which genes expressed as nerve growth factors are included in the construct; and diabetes, for which insulin producing genes may be included in the construct.

Genetic diseases caused by defects in the genetic pathways effecting DNA repair, e.g., ataxia telangiectasia, xeroderma pigmentosum, are treated by the introduction of genes such as ERCC-1 or XRCC-1.

Alth ugh the practice of this invention requires exp sure t radiati n, an agent which in itself may advers ly affect c lls, the d se is relativ ly low, administered for brief periods f time, and focused. For many f the dis as s and conditions for which this

invention is appropriate, radiation treatment is standard, and practice of this invention will reduce th necessary dose, which reduces risk of the radiation treatment per se. For diseases which usually do not require radiation, use of radiation in the methods described in this invention will replace another therapy. Decision on use of this invention will be based on a risk/benefit analysis.

Definitions

Effector Gene - a gene whose expression product produces the desired effect in the recipient cells and target tissues.

Enhancer Gene or Element - a *cis*- acting nucleic acid sequence that increases the ulitization of some eukaryotic promoters, and can function in either orientation and in any location (upstream or downstream) relative to the promoter.

LAK Cells - lymphocyte activated killer cells.

Promoter - a region of DNA involved in binding RNA polymerase to initiate transcription.

Reporter Gene - a gene whose expression product is readily detectable and serves as a marker for the expression of induction.

Structural Gene - a gene coding for a protein with an effector function. This protein might be an enzyme, toxin, ligand for a specific receptor, receptor, nucleic acid binding protein or antigen. The protein could also serve as a reporter to monitor induction by ionizing radiation. The gene coding for these proteins could be derived from eukaryot s r prokary tes.

Other obj cts and advantages of the inv ntion will become apparent upon r ading the following detailed description and up n reference to the drawings in which:

FIG. 1. A schematic drawing of th basic genetic construct comprising a radiation sensitive promoter driving an effector gene.

FIG. 2. A schematic drawing of a more complex genetic construct than that shown in FIG. 1, comprising an "amplification system."

FIG. 3. A schematic drawing comprising the basic system of a retroviral mode of infection of a genetic construct into a cell.

FIG. 4. Effects of irradiation on TNF-α gene expression.

FIG. 5. Influence of TNF-α on radiation lethality of TNF-α-producing human sarcomas and TNF-α-nonproducing human tumor cells.

FIG. 6. Effects of ionizing radiation on c-*jun* RNA levels in human HL-60 cells.

FIG. 7. Effects of ionizing radiation on c-*jun* RNA levels in U-937 cells and in human AG-1522 diploid fibroblasts.

FIG. 8. Effects of ionizing radiation on rates of c-*jun* gene transcription.

FIG. 9. Effects of cycloheximide on c-*jun* mRNA lev ls in ionizing radiati n-treated HL-60 cells.

FIG. 10. Effects of ionizing radiation on C-*fos* and *jun-B* mRNA lev ls in HL-60 cells.

FIG. 11. Effects of dose rate on th induction of *c-jun* expression by ionizing radiation.

While the invention is susceptible to various modifications and alternative forms, a specific embodiment thereof has been shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that it is not intended to limit the invention to the particular forms disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

This invention relates to methods and compositions of controlling expression of a gene by exposure of a construct, including the gene, to ionizing radiation. The genes to be controlled are preferably incorporated within a genetic construct which includes a region which is sensitive to ionizing radiation. A schematic diagram of such a construct is shown in FIG. 1 wherein an enhancer-promoter region 10 of a radiation response gene, e.g., *c-jun*, drives 16 the expression of a structural gene, e.g., a reporter-effector gene such as TNF 14. The product of the structural gene expression is then capable of acting on a cell which has incorporated it, to produce a desired effect on the cell.

A more complex genetic construct is shown schematically in FIG. 2. In FIG. 2A, a region 20 comprising an enhancer-promoter of a radiation responsiv gene, is coupled to, and drives 28 the expr ssi n of, a DNA binding domain 26, e.g., of a LAC repressor gen , and a gene 24 producing a transcription factor, e.g., from VP16. The chimeric protein resulting from th expression of that fusion gen , 40, 42 is capable of binding t a

DNA sequenc 30 illustrated in FIG. 2B. Binding of this sequence by the transcription factor 40, 42 activates 38 a structural gene 36, e.g., a reporter-effector gene such as TNF. A "minimal promoter" 32 containing CCAAT and th TATA boxes, e.g., from the c-*fos* oncogene, is placed between the binding sequence 30 and the genes 36 to be expressed. The gene product 34 is capable of acting on a cell which has incorporated the genetic constructs, to produce a desired effect.

An example showing details of the multiple gene form of genetic construct is shown in FIG. 2. This figure is predicated on strong induction of the c-*jun* gene in various different cell types by ionizing radiation at a transcriptional level. A large piece of 5′ genomic sequence from the *jun* gene is ligated to an appropriate reporter such as β-galactosidase. Such a construct is then transfected into a recipient cell and checked for radiation responsiveness. Various truncations of this initial large 5′ piece may be used.

Methods of incorporating constructs into recipient cells comprise electroporation, lipofection, and viral infection. This latter method comprises a SIN (self-inactivating virus) with two LTR's 50, 56. Nestled between the LTR's is a genetic construct comprising a radiation sensitive element 52 and a structural gene region 54. A U3 enhancer deletion is shown at 58.

Examples of elements used for the constructs follow.

Radiation Regulates TNF-α Expression

Combinations f tum r necrosis factor α (TNF-α), a polyp ptid mediator of the c llular immune response with pleiotropic activity, and radiation produc synergistic

effects and are us ful f r clinical cancer th rapy. TNF-α acts directly on vascular endothelium to increase the adhesion of leukocytes during the inflammatory process (Bevelacqua, et al., 1989). This *in vivo* response to TNF-α was suggested to be responsible for hemorrhagic necrosis and regression of transplantable mouse and human tumors (Carswell, 1975). TNF-α also has a direct effect on human cancer cell lines *in vitro*, resulting in cell death and growth inhibition (Sugarman, et al., 1985; Old, 1985). The cytotoxic effect of TNF-α correlates with free-radical formation, DNA fragmentation, and microtubule destruction (Matthews, et al., 1988; Rubin, et al., 1988; Scanlon, et al., 1989; Yamauchi, et al., 1989; Matthews, et al., 1987; Neale, et al., 1988). Cell lines that are resistant to oxidative damage by TNF-α also have elevated free-radical buffering capacity (Zimmerman, et al., 1989; Wong, et al., 1988).

TNF-α causes hydroxyl radical production in cells sensitive to killing by TNF-α (Matthews, et al., 1987). Cell lines sensitive to the oxidative damage produced by TNF-α have diminished radical-buffering capacity after TNF-α is added (Yamauchi, et al., 1989). Lower levels of hydroxyl radicals have been measured in cells resistant to TNF-α cytotoxicity when compared with cells sensitive to TNF-α killing (Matthews, et al., 1987).

Tumor necrosis factor α is increased after treatment with x-rays in certain human sarcoma cells. The increase in TNF-α mRNA is accompanied by the increased production of TNF-α protein.

The induction f a cytot xic pr tein by exposure of c lls containing the TNF gen to x-rays was suspected when m dium d canted from irradiat d cultures of some human sarcoma cell lines was f und t be cytotoxic to

th s cells as well as to other tumor c ll lines. The level of TNF-α in the irradiated tumor cultures was elevated over that of nonirradiated cells when analyzed by the ELISA technique (Saribon, et al., 1988). Subsequent investigations showed that elevated TNF-α protein after irradiation potentiates x-ray killing of cells by an unusual previously undescribed mechanism (se Example 1).

FIG. 4 illustrates the effects of irradiation on TNF-α gene expression. RNA from untreated cells (control) and irradiated cells was size-fractionated and hybridized to ^{32}P-labeled TNF-α cDNA (STSAR-13) and PE4 plasmid containing TNF-α cDNA (STSAR-48). Autoradiograms showed increased expression of TNF-α mRNA 3 hr after irradiation in cell line STSAR-13 and at 6 hr in cell line STSAR-48. 7S RNA was hybridized to show the pattern for equally loaded lanes. The conclusion from these results is that there is increased TNF-α gene expression after radiation.

The next question was what the effects of TNF-α and radiation would be on cell killing. FIG. 5 exhibits the influence of TNF-α on radiation lethality of TNF-α-producing human sarcomas and TNF-α-nonproducing human tumor cells. The solid lines indicate the effects of radiation alone, and the dashed lines indicate the effects of both TNF-α and irradiation. Representative survival data for cell line STSAR-33 are shown in the graph to the left, A. The lower dashed line represents survival of cells with TNF-α at 1000 units/ml, corrected f r a plating efficiency (PE) of 30%. The survival of human epithelial tumor cells (SQ-20B) irradiated with TNF-α (10 units/ml and 1000 units/ml) is shown in the middl graph, B. Survival data for SQ-20B show an additive eff ct of TNF-α (1000 units/ml). Survivals with

TNF-α are corrected for 85% killing with TNF-α alone. Radiation survival data for HNSCC-68 is shown in the graph to the right, C. A nonlethal dose of TNF-α (10 units/ml) was added 24 hr before irradiation.

As can be seen from these results and from information discussed in EXAMPLE 1, the tumor necrosis factor α is increased after treatment with x-rays. Both mRNA and TNF-α proteins were increased.

Although DNA-damaging agents other than ionizing radiation have been observed to induce expression of variety of prokaryotic and mammalian genes, the TNF-α gene is the first mammalian gene found to have increased expression after exposure to ionizing radiation. This gene is not categorized as a DNA repair gene.

To determine the mechanisms responsible for regulation of *c-jun* gene expression by ionizing radiation, run-on transcriptional assays were performed in isolated nuclei. The action gene was constitutively transcribed in untreated HL-60 cells as a positive control (FIG. 8).

Negative control was provided by the β-globin gene transcript. As shown in FIG. 8, a low level of *c-jun* transcription was detectable in HL-60 untreated by radiation. Dramatic increased transcription (7.2 fold) occurred after exposure to ionizing radiation. The conclusion from this study was that ionizing radiation induced *c-jun* expressi n, at l ast in part by a transcriptional mechanism.

FIG. 9 illustrat s the effects of cycloheximide on *c-jun* mRNA levels in i nizing radiation treat d HL-60 cells. The columns headed XRT shows xpression of mRNA

after 20 Gy radiation exposure of the cells. In th columns CHX, cycloheximide has been added. The additive effects of CHX and CHX/XRT are a 3.6 fold increased expression compared to XRT alone.

Effects of cycloheximide on c-*jun* mRNA levels in ionizing radiation-treated HL-60 cells. HL-60 cells were treated with 20 Gy of ionizing radiation (XRT) and/or 5 μg of cycloheximide (CHX) per ml. Total cellular RNA (20 μg per lane) was isolated after 1, 3 and 6 h and analyzed by hybridization to the ^{32}P-labeled c-*jun* or actin probe.

FIG. 10. Effects of ionizing radiation on C-*fos* and *jun-B* mRNA levels in HL-60 cells. (A) HL-60 cells wer treated with varying doses of ionizing radiation (XRT) or 32 nM 12-*O*-tetradecanoylphorbol 13-acetate (TPA; positive control) for 3 h. Total cellular RNA (20 μg) was hybridized to the ^{32}P-labeled c-*fos* probe. (B) HL-60 cells were treated with 20 Gy of ionizing radiation. Total cellular RNA (20 μg per lane) was isolated at the indicated times and analyzed by hybridization to the ^{32}P-labeled *jun-B* probe.

FIG. 11. Effects of dose rate on the induction of c-*jun* expression by ionizing radiation. HL-60 cells were treated with 10 or 20 Gy of ionizing radiation at the indicated dose rates. After 3 h, total cellular RNA (20 μg) was isolated and hybridized to the ^{32}P-labelled c-*jun* probe.

Targeting Tissues for Incorporation of a Genetic Construct Responsive to Ionizing Radiation

Depending on the application in question, the recipi nt c lls ar targeted in various ways. In an xemplary embodiment, LAK cells which tend to home in on

the tumor site in question with som degree f prefer nc though as is well known, they will also distribute themselves in the body in other locations, may be used to target tumors. Indeed, one of the most important advantages of the radiation inducible system is that only those LAK cells, which are in the radiation field will be activated and will have their exogenously introduced lymphokine genes activated. Thus, for the case of LAK cells, there is no particular need for any further targeting. In other applications, the appropriate cells in question have had appropriate genes from monoclonal antibodies introduced in them or appropriate antibodies expressed on their cell surface by other means such as by cell fusion. These monoclonal antibodies, for example, are targeted towards specific cells in the body and thus allow the recipient cells to home in on that particular region so that then radiation could be used for the activation of the appropriate toxins within them. This enables local delivery of the "drug," wherein the "drug" is defined as the expression product of the genes within the radiation responsive genetic construct. Illustrative embodiments of types of radiation inducible constructs and their applications are presented in Table 1 and EXAMPLE 4.

TABLE 1: ILLUSTRATIVE EMBODIMENTS OF TYPES OF RADIATION INDUCIBLE GENETIC CONSTRUCTS AND THEIR USES

Action of Expression Products of Genes in the Construct	Examples of Structural Genes Used in the Construct	Applications to Diseases, Conditions and Tissues
Kill tumor cells	Toxins TNF Growth Factors (IL-1-6 PDGF, FGF)	Solid and Hematologic Malignancies
Protect normal tissues from radiation and other cytotoxins during cancer therapy	Lymphokines GCSF, CMCSF Erythropoietin	Solid and Hematologic Malignancies, Aplastic Anemic
Inhibit Metastasis	NM23	Cancer Metastasis
Tumor Suppressor Gene Products	Rb p53	Prevention of Malignancy Following Standard Radiotherapy and Chemotherapy
Radiosensitization Chemosensitization (enhance routine treatment effects)	TNF	Solid and Hematologic Malignancies
Correct Defects in Clotting Factors	Factor 8	Clotting Disorders
Introduce Anticlotting Factors	Streptokinase Urokinase	Myocardial Infarction, CNS Thrombosis, Pheripheral Thrombosis
Correct Defects Characterizing Hemoglobinopathy	Normal Hemoglobin	Sickle Cell Anemia
Correct Deficienceis Leading to Neurodeg nerative Dis ase	Nerve Growth Factor	Alzheimer's Disease
Provide Tr atment Comp nent for Diabetes	Insulin	Diab tes

Action of Expression Products of Genes in the Construct	Examples of Structural Genes Used in the Construct	Applications to Diseases, Conditions and Tissues
Disease of DNA Repair Abnormalities	ERCC-1, XRCC-1	Ataxia Telangiectasia Xeroderma Pigmentosum

EXAMPLES

EXAMPLE 1
Increased Tumor Necrosis Factor α mRNA After Cellular Exposure to Ionizing Radiation

A. Protein Products

To investigate TNF-α protein production after x-irradiation, the levels of TNF-α in the medium of human tumor cell lines and fibroblasts were quantified by the ELISA technique (Saribon, et al., 1988) before and after exposure to 500-cGy x-rays (Table 1). Five of 13 human bone and soft tissue sarcoma cell lines (STSAR-5, -13, -33, -43, and -48) released TNF-α into the medium after irradiation, whereas TNF-α levels were not elevated in supernatant from normal human fibroblast cell lines (GM-1522 and NHF-235) and four human epithelial tumor cell lines (HN-SCC-68, SCC-61, SCC-25, and SQ-20B) after exposure to radiation. The assay accurately measures TNF-α levels between 0.1 and 2.0 units per ml (2.3 x 10^6 units/mg) (Saribon, et al., 1988). Tumor cell line STSAR-13 produced undetectable amounts of TNF-α before x-irradiation and 0.35 units/ml after x-ray exposure. Cell lines STSAR-5 and -33 responded to x-irradiation with increases in TNF-α concentrations of >5- to 10-f ld; how ver quantities above 2 units/ml exceeded the range of the assay (Sarib n, et al., 1988). Cell lines STSAR-43 and -48 demonstrated incr ases in TNF-α f 1.5- to 3-f ld (Tabl 1). TNF-α protein in the medium was first

elevated at 20 hr after x-ray treatm nt, reached maximal levels at 3 days, and remained elevated beyond 5 days. Furthermore, supernatant from irradiated, but not control STSAR-33, was cytotoxic to TNF-α-sensitive cell line SQ-20B.

TABLE 2: PRODUCTION OF TNF-A IN HUMAN SARCOMA CELL LINES

Cell Line	Origin	TNF-α level, units/ml, Control	X-ray
STSAR-5	MFH	0.4	>2.0
STSAR-13	Liposarcoma	0.0	0.34
STSAR-33	Ewing sarcoma	0.17	>2.0
STSAR-43	Osteosarcoma	0.41	1.3
STSAR-48	Neurofibrosarcoma	0.28	0.43

TNF-α levels were measured in medium from confluent cell cultures (control) and in irradiated confluent cells (x-ray). TNF-α levels increased as measured by the ELISA technique. MFH, malignant fibrous histiocytoma.

B. RNA Analysis.

Increased levels of TNF-α mRNA were detected in the TNF-α-producing sarcoma cell lines after irradiation relative to unirradiated controls (FIG. 4). For example, TNF-α transcripts were present in unirradiated STSAR-13 and -48 cell lines. TNF-α mRNA levels in cell line STSAR-13 increased by >2.5-fold as measured by densitometry 3 hr after exposure to 500 cGy and then declined to baseline levels by 6 hr (FIG. 4). These transcripts increased at 6 hr after irradiation in cell line STSAR-48, thus indicating some heterogeneity between cell lines in terms of the kinetics f TNG-α gene expr ssion (FIG. 4). In contrast, irradiation had no detectable effect on 7S RNA levels (FIG. 4) or expr ssion of the polymeras β g n .

C. Interaction Between TNF-α and X-Irradiation.

To investigate the influence of TNF-α on radiation-induced cytotoxicity in TNF-α-producing cell lines, recombinant human TNF-α was added to cultures before irradiation (FIG. 5). Recombinant human TNF-α (1000 units/ml) (2.3×10^6 units/mg) was cytotoxic to four of five TNF-α-producing sarcomas (STSAR-5, -13, -33, and -43). The plating efficiency (PE) was reduced by 60-90% at 1000 units/ml in these lines. Radiation-survival analysis of cell line STSAR-33 was performed with TNF-α (10 units/ml). The radiosensitivity (D_0), defined as th reciprocal of the terminal slope of the survival curves was 80.4 cGy for cell line STSAR-33. When TNF-α was added 20 hr before irradiation, the D_0 was 60.4 cGy. Surviving fractions were corrected for the reduced PE with TNF-α. Thus, the interaction between TNF-α and radiation in STSAR-33 cells was synergistic (Dewey, 1989). Sublethal concentrations of TNF-α (10 units/ml) enhanced killing by radiation in cell line STSAR-33, suggesting a radiosensitizing effect of TNF-α The surviving fraction of cell line STSAR-5 at 100-700 cGy was lower than expected by the independent killing of TNF-α and x-rays, although the D_0 values were similar. Thus, the interaction between TNF-α and radiation is additive (Dewey, 1979) in STSAR-5 cells. Cell lines STSAR-13 and STSAR-43 were independently killed with x-rays and TNF-α, and no interaction was observed.

To determine the possible interactions between TNF-α and x-rays in non-TNF-α producing cells, human epithelial tum r cells (SQ-20B and HNSCC-68) were irradiated 20 hr after TNF-α was added. Thes cell lin s do not product TNF-α in resp ns to i nizing radiation. TNF-α (1000 units/ml) was cyt toxic t SQ-20B and SCC-61 cells, reducing the PE by 60-80%. The radiation survival f SQ-20B cells with and without TNF-α is shown in FIG. 5. The

D_0 for cell line SQ-20B is 239 cGy. With TNF-α (1000 units/ml) added 24 hr before x-rays, the D_0 was 130.4 cGy. Therefore, a synergistic interaction (Dewey, 1979) between TNF-α and x-rays was demonstrated in this cell line. TNF-α added after irradiation did not enhance c ll killing by radiation in cell lines SQ-20B. Nonlethal concentrations of TNF-α (10 units/ml) resulted in enhanced radiation killing in cell line HNSCC-68 (FIG. 5), providing evidence that TNF-α may sensitize some epithelial as well as mesenchymal tumor cell lines to radiation.

The following specific methods were used in Example 1.

Cell Lines. Methods of establishment of human sarcoma and epithelial cell lines have been described (Weichselbaum, et al., 1986; 1988). Culture medium for epithelial tumor cells was 72.5% Dulbecco's modified Eagle's medium/22.5% Ham's nutrient mixture F-12 [DMEM/F-12 (3:1)]5% fetal bovine serum (FBS), transferrin at 5 μg/ml/10^{-10} M cholera toxin/1.8 x 10^{-4} M adenine, hydrocortisone at 0.4 μg/ml/2 x 10^{-11} M triodo-L-thyronine/penicillin at 100 units/ml/streptomycin at 100 μg/ml. Culture medium for sarcoma cells was DMEM/F-12 (3:1)/20% FBS, penicillin at 100 units/ml/streptomycin at 100 μg/ml.

TNF-α Protein Assay. Human sarcoma cells were cultured as described above and grown to confluence. The medium was analyz d f r TNF-α 3 days after feeding and again 1-3 days after irradiation. Thirt en established human sarcoma cell lines were irradiated with 500-c ntigray (cGy) x-rays with a 250-kV Maxitron g nerator (Weichs lbaum, et al., 1988). TNF-α was measured by ELISA with two monocl nal antibodies that had distinct

epitopes for TNF-α protein (Saribon, et al., 1988); the assay detects TNF-α from 0.1 to 2.0 units/ml.

RNA Isolation and Northern (RNA) Blot Analysis. Total cellular RNA was isolated from cells by using the guanidine thiocyanate-lithium chloride method (Cathala, et al., 1983). RNA was size-fractionated by formaldehyde-1% agarose gel electrophoresis, transferred to nylon membranes (GeneScreen*Plus*, New England Nuclear), hybridized as previously described to the 1.7-kilobase (kb) *Bam*HI fragment of the PE4 plasmid containing TNF-α cDNA (19, 23), and autoradiographed for 16 days at -85·C with intensifying screens. Northern blots were also hybridized to 7S rRNA and β-polymerase plasmids as described (Fornace, et al., 1989). Ethidium bromide staining revealed equal amounts of RNA applied to each lane. RNA blot hybridization of TNF-α was analyzed after cellular irradiation with 500 cGy. Cells were washed with cold phosphate-buffered saline and placed in ice at each time interval. RNA was isolated at 3, 6, and 12 hr after irradiation.

Treatment of Cells with X-Irradiation and TNF-α. Exponentially growing cells were irradiated by using a 250-kV x-ray generator. The colony-forming assay was used to determine cell survival (Weichselbaum, et al., 1988). The multitarget model survival curves were fit to a single-hit multitarget model [$S = 1 - (-e^{-D|D0})^n$]. Concentrations of recombinant human TNF-α (10 units/ml) (2:3 x 10^6 units/mg) and (1000 units/ml) (Asahi Chemical, New York) were added 24 hr bef re irradiation.

EXAMPLE 2

Increased c-*jun* Expr ssion After Exposur to I nizing Radiati n

Th following m thods w re used in this example.

Radiation Regulates c-*jun* Expression

Another embodiment of a genetic construct derives from the c-*jun* protooncogene and related genes. Ionizing radiation regulates expression of the c-*jun* protooncogene, and also of related genes c-*fos* and *jun*-β. The protein product of c-*jun* contains a DNA binding region that is shared by members of a family of transcription factors. Expression level after radiation is dose dependent. The c-*jun* gene encodes a component of the AP-1 protein complex and is important in early signaling events involved in various cellular functions. AP-1, the product of the protooncogene c-*jun* recognizes and binds to specific DNA sequences and stimulates transcription of genes responsive to certain growth factors and phorbol esters (Bohmann, et al., 1987; Ang l, et al., 1988). The product of the c-*jun* protooncogene contains a highly conserved DNA binding domain shared by a family of mammalian transcription factors including *jun*-β, *jun*-D, c-*fos*, *fos*-β, *fra*-1 and the yeast GCN4 protein.

In addition to regulating expression of the c-*jun* gene, c-*jun* transcripts are degraded posttranscriptionally by a labile protein in irradiated cells. Posttranscriptional regulation of the gene's expression is described in Sherman, et al., 1990.

Contrary to what would be expected based on previous DNA damage and killing rates for other agents, decreasing th dose rate, for exampl , from 14.3 Gy/min to 0.67 Gy/min. was ass ciated with increased inducti n of c-*jun* transcripts.

FIG. 6. Effects f i nizing radiation on c-*jun* RNA levels in human HL-60 cells. (A) Northern blot analysis of total cellular RNA levels was performed in HL-60 cells after treatment with 20 Gy of ionizing radiation (XRT). Hybridization was performed using a ^{32}P-labeled c-*jun* or actin DNA probe. (B) HL-60 cells were treated with the indicated doses of ionizing radiation. RNA was isolated after 3 hours and hybridizations were performed using ^{32}P-labeled c-*jun* or β-actin DNA probes. The column labelled HL-60 represents RNA from untreated cells.

Maximum c-*jun* mRNA levels were detectable after 50 Gy of ionizing radiation (FIG. 6B).

Similar kinetics of c-*jun* induction were observed in irradiated human U-937 monocytic leukemia cells (FIG. 7A) and in normal human AG-1522 diploid fibroblasts (FIG. 7B). Treatment of AG-1522 cells with ionizing radiation was also associated with the appearance of a minor 3.2-kb c-*jun* transcript.

Cell Culture. Human HL-60 promyclocytic leukemia cells, U-937 monocytic leukemia cells (both from American Type Culture Collection), and AG-1522 diploid foreskin fibroblasts (National Institute of Aging Cell Repository, Camden, NJ) were grown in standard fashion. Cells were irradiated using either Philips RT 250 accelerator at 250 kV, 14 mA equipped with a 0.35-mm Cu filter or a Gammacell 1000 (Atomic Energy of Canada, Ottawa) with a ^{137}Cs source emitting at a fixed dose rate of 14.3 Gy/min as determin d by dosimetry. Control cells were exposed t the same conditions but not irradiated.

Northern Blot Analysis. Total cellular RNA was is lated as describ d (29). RNA (20 µg per lane) was s parat d in an agarose/f rmaldehyd gel, transferred to

a nitr c llulos filter, and hybridized to the f llowing ^{32}P-labeled DNA probes: (i) the 1.8-kilobase (kb) *BamHI/EcoRI c-jun* cDNA (30); (ii) the 0.91-kb *Sca I/Nco* I *c-fos* DNA consisting of exons 3 and 4 (31); (iii) the 1.8-kb *EcoRI jun-B* cDNA isolated from the p465.20 plasmid (32); and (iv) the 2.0-kb *PstI* β-actin cDNA purified from pA1 (33). The autoradiograms were scanned using an LKB UltroScan XL laser densitometer and analyzed using the LKB GelScan XL software package. The intensity of *c-jun* hybridization was normalized against β-actin expression.

Run-On Transcriptional Analysis. HL-60 cells were treated with ionizing radiation and nuclei were isolat d after 3 hours. Newly elongated ^{32}P-labeled RNA transcripts were hybridized to plasmid DNAs containing various cloned inserts after digestion with restriction endonulceases as follows: (i) the 2.0-kb *Pst* I fragment of the chicken β-actin pA1 plasmid (positive control); (ii) the 1.1-kb *BamHI* insert of the human β-globin gene (negative control, ref.34); and (iii) the 1.8-kb *BamHI/EcoRI* fragment of the human *c-jun* cDNA from the pBluescript SK(+) plasmid. The digested DNA was run in a 1% agarose gel and transferred to nitrocellulose filters by the method of Southern. Hybridization was performed with 10^7 cpm of ^{32}P-labeled RNA per ml of hybridization buffer for 72 h at 42°C. Autoradiography was performed for 3 days and the autoradiograms were scanned as already described.

EXAMPLE 3

Radiation Induced Transcription of *JUN* and *EGR1*

Th re was increas d mRNA expression for different class s of immediate early response to radiati n genes (*JUN, EGR1*) within 0.5 t 3 hours f llowing cellular x-irradiation. Pr incubati n with cycloheximide was

ass ciated with superinduction of *JUN* and *EGR1* in x-irradiated cells. Inhibition of protein kinase C (PKC) activity by prolonged stimulation with TPA or the protein kinase inhibitor H7 prior to irradiation attenuated the increase in *EGR1* and *JUN* transcripts. These data implicated *EGR1* and *JUN* as signal transducers during th cellular response to radiation injury and suggested that this effect is mediated in part by a protein kinase C (PKC) dependent pathway.

JUN homodimers and *JUN/FOS* heterodimers regulate transcription by binding to *AP1* sites in certain promoter regions (Curran and Franza, 1988). The *JUN* and *FOS* genes are induced following x-ray exposure in human myeloid leukemia cells suggests that nuclear signal transducers participate in the cellular response to ionizing radiation.

EGR1 (also known as zif/268, NGFI-1, Krox-24, TIS-8) (Christy, et al., 1988; Milbrant, 1987; Lemaire, et al., 1988; Lim, et al., 1987) encodes a nuclear phosphoprotein with a Cys_2-His_2 zinc-finger motif which is partially homologous to the corresponding domain in the Wilms' tumor susceptibility gene (Gessler, 1990). The *EGR1* protein binds with high affinity to the DNA sequence CGCCCCCGC in a zinc-dependent manner (Christy and Nathans, 1989; Cao, 1990). *EGR1* represents an immediate early gene which is induced during tissue injury and participates in signal transduction during cellular proliferation and differentiation.

The *EGR1* and *JUN* genes are rapidly and transiently expressed in th abs nce of de novo pr tein synthesis after ionizing radiation exposure. *EGR1* and *JUN* are m st lik ly involved in signal transduction following x-irradiation. Down regulati n of PKC by TPA and H7 is

associat d with attenuation of *EGR1* and *JUN* gene induction by ionizing radiation, implicating activation of PKC and subsequent induction of the *EGR1* and *JUN* genes as signaling events which initiate the mammalian cell phenotypic response to ionizing radiation injury.

Control RNA from unirradiated cells demonstrated low but detectable levels of *EGR1* and *JUN* transcripts. In contrast, *EGR1* expression increased in a dose dependent manner in irradiated cells. Levels were low but detectable after 3 Gy and increased in a dose dependent manner following 10 and 20 Gy. Twenty Gy was used in experiments examining the time course of gene expression so that transcripts were easily detectable. Cells remained viable as determined by trypan dye exclusion during this time course. A time dependent increase in *EGR1* and *JUN* mRNA levels was observed. SQ-20B cells demonstrated coordinate increases in *EGR1* and *JUN* expression by 30 minutes after irradiation that declined to baseline within 3 hours. In contrast, *EGR1* transcript levels were increased over basal at 3 hours while *JUN* was increased at one hour and returned to basal at 3 hours in AG1522. *JUN* levels were increased at 6 hours in 293 cells while *EGR1* was increased at 3 hours and returned to basal levels by 6 hours.

To determine whether *EGR1* and *JUN* participated as immediate early genes after x-irradiation, the effects of protein synthesis inhibition by CHI were studied in cell lines 293 and SQ-20B after x-ray exposure. CHI treatment alone resulted in a low but detectable increase in *EGR1* and *JUN* transcripts n rmalized to 7S. In the abs nce of CHI, the l v l of *EGR1* and *JUN* expression returned to baseline. In contrast, SQ-20B cells pr treated with CHI dem nstrat d persistent elevation f *EGR1* at 3 hours and 293 cells dem nstrated persistent elevation of *JUN* mRNA

at 6 hours after irradiation thus indicating superinduction of these transcripts.

mRNA levels of transcription factors *EGR1* and *JUN* increased following ionizing radiation exposure in a time and dose dependent manner. The potential importance of the induction of *EGR1* and *JUN* by ionizing radiation is illustrated by the recent finding that x-ray induction of the PDGF alpha chain stimulates proliferation of vascular endothelial cells (Witte, et al., 1989). PDGF has AP-1 and *EGR1* binding domains while TNF has elements similar to AP-1 and *EGR1* target sequences (Rorsman, et al., 1989; Economou, et al., 1989). X-ray induction of PDGF and TNF appears to be regulated by *EGR1* and *JUN*.

The following is a method used in EXAMPLE 3:

Kinase Inhibitors

Cell line SQ-20B was pretreated with 1 μM TPA for 40 hours to down regulate PKC and then stimulated with TPA, serum, or x-ray (20 Gy). Controls included x-ray without TPA pretreatment, TPA (50 nM) without TPA pretreatment and untreated cells. RNA was isolated after one hour and hybridized to *EGR1*. SQ-20B cells were preincubated with 100 μM H7 (1-(5-isoquinolinylsulfonyl)-2-methyl piperazine) or 100 μM HA1004 (N-[2-methyl-amino] ethyl)-5-isoquino-linesulfonamide) Seikagaku America, Inc., St. Petersberg, FL) for 30 minutes or TPA pretreatment (1 μM) for 40 hours and f llowed by exp sure to 20 Gy x-irradiation. RNA was extracted one h ur after irradiation. Positive control cells treated under th same conditions but in the absence of inhibitor also received 20 Gy, while negativ control c lls r ceived neither H7 nor X-ray. RNA was extracted at on h ur

after 20 Gy without inhibitor. Northern blots w r hybridized to *EGR1* or 7S. 293 cells pretreated with the above inhibitors were irradiated, RNA was extracted aft r 3 hours and the Northern blot was hybridized to *JUN* and 7S probes.

EXAMPLE 4

Protocol for Treatment of Head and Neck Cancer with X-ray Induced TNF and Therapeutic X-rays

For treatment of patients with head and neck cancer, the following steps are followed:

1. Prepare a genetic construct according to the general scheme illustrated in FIGS. 1 or 2.

This construct comprises AP-1 as the element which is responsive to x-rays, coupled to a sequence of DNA to which the lac repressor binds, and to the gene for the tumor necrosis factor. This construct is designated "construct A" for purposes of this example.

2. "Construct A" is put into a retrovirus that is self-inactivating (see FIG. 3).

3. Lymphokine activated killer (LAK) cells are infected with the retrovirus bearing "construct A." Th cells are to be directed against the malignant cells in the head and neck.

4. The lymphocytes are infused into the patient t be treated.

5. The head and neck regi n is irradiated.

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Ref r nc 59. Wong, G.H.W., Elwell, J.H., Oberly, L.H., Goeddel, D.V. (1989) *Cell* 58:923-931.

Reference 60. Yamuchi, N., Karizana, H., Watanabe, H., Neda, H., Maeda, M. and Nutsu, Y. (1989) *Cancer Res*. 49:1671-1675.

Reference 61. Zimmerman, R.J., Chan, A. and Leadon, S.A. (1989) *Cancer Res*. 49:1644-1648.

CLAIMS:

1. A method for destroying, altering, or inactivating cells within a tissue, said method comprising:

(a) preparing a genetic construct comprising a radiation responsive enhancer-promoter region and a region comprising at least one structural gene which is controlled by the enhancer-promoter;

(b) delivering the construct into cells in the tissue or into cells that migrate to the tissue; and

(c) exposing the tissue to ionizing radiation to induce the expression of the structural gene.

2. The method of claim 1 wherein the radiation responsive enhancer-promoter region is derived from at least one of the following genes: c-*jun*, AP-1 and tumor necrosis factor.

3. The method of claim 1 wherein the structural gene region comprises genes whose expression comprises at least one of the following: tumor necrosis factor, ricin, and streptokinase.

4. The method of claim 1 wherein the structural gene region comprises a DNA binding domain, a repressor gene, a binding region for the repressor and a structural gene.

5. The method of claim 1 wherein the ionizing radiation is delivered at a low dose.

6. The method of claim 5 wherein the dose is in the range of 150 to 300 rads.

7. The method of claim 6 wherein the dose is about 200 rads.

8. The method of claim 1 wherein the tissue is a disease-related tissue.

9. The method of claim 8 wherein the disease-related tissue comprises a tumor.

10. The method of claim 8 wherein the disease-related tissue comprises a blood clot.

11. The method of claim 1 wherein the tissue is characterized by a metabolic deficiency.

12. A method of treating a disease comprising:

(a) preparing a g netic construct comprising a promoter which is inducible by ionizing radiation, and at least one structural gene;

(b) delivering the genetic construct to the disease-related tissue so that the cells within the tissue incorporate the construct; and

(c) exposing the tissue to ionizing radiation to induce the expression of the gene.

13. The method of claim 12 wherein the genetic construct comprises the c-*jun* promoter and the structural gene for the tumor necrosis factor.

14. The method of claim 12 wherein the structural gene which is capable of being activated by a transcription factor, the expression of which is under the control of the radiation inducible promoter.

15. The method of claim 14 wherein the structural gene comprises a fusion gene comprising a lac repressor, a DNA binding domain of a LAC repressor, a VP16 actuation domain, a LAC repressor binding sequence, and a reporter-effector gene which is activated by a lac repressor.

16. The method of claim 12 wherein the target tissue is a tumor and the disease to be treated is cancer.

17. The method of claim 12 wherein the target tissue is a blood clot and the disease to be treated is myocardial infarction of the myocardium, brain, lung or other tissues.

18. A method of causing the expression within a host tissue of a structural gene, said method comprising:

(a) delivering *in vitro* the vector comprising a radiation responsive enhancer-promoter region and a structural gene to cells derived from the host tissue;

(b) reintroducing the cells from the host tissue to the host; and

(c) activating the genes within the vector by exposure of the tissue to radiation.

19. The method of claim 18 wherein the radiation exposure is delivered at a dose of about 200 rads.

20. A method for destroying, altering, or inactivating cells, comprising:

(a) incorporating into the cells a genetic construct which comprises genes whose expression is inducible by ionizing radiation and whose products are capable of destroying, altering or inactivating the cells; and

(b) exposing the construct containing cells to ionizing radiation to induce the expression of genes in the genetic construct.

21. A gen tic construct comprising a promoter which is inducible by ionizing radiation and a structural gene.

22. The genetic construct of claim 21 wherein the structural gene is a mammalian gene.

23. The genetic construct of claim 21 further defined as having the inducible promoter 5′ to the structural gene.

24. The genetic construct of claim 21 comprising the promoter for the tumor necrosis factor and the structural gene for the tumor necrosis factor.

25. The genetic construct of claim 21 wherein the structural gene comprises a plant toxin gene.

26. A vector comprising a radiation responsive enhancer-promoter region and a structural gene.

27. A vector comprising a radiation responsive promoter which controls the expression of a transcription factor, said transcription factor being capable of activating a genetic construct comprising a structural gene.

28. A method for controlling structural gene expression, said method comprising:

(a) preparing a genetic construct comprising the structural gene to be expressed and a mammalian genetic promoter-enhancer region which is inducible by ionizing radiation;

(b) exposing the genetic construct to ionizing radiation at a dose sufficient to induce expression of the structural gene.

29. The method of claim 28 wherein the promoter-enhancer region is derived from the genes coding for the c-*jun* or TNF-α proteins.

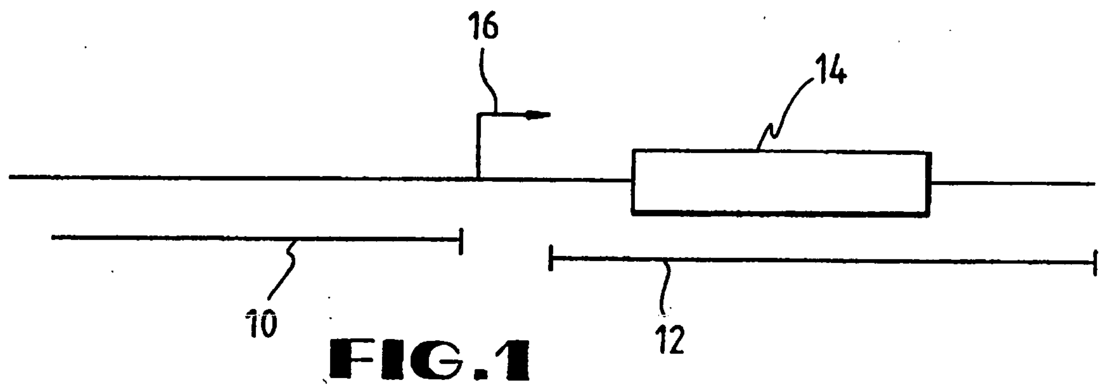


FIG.1

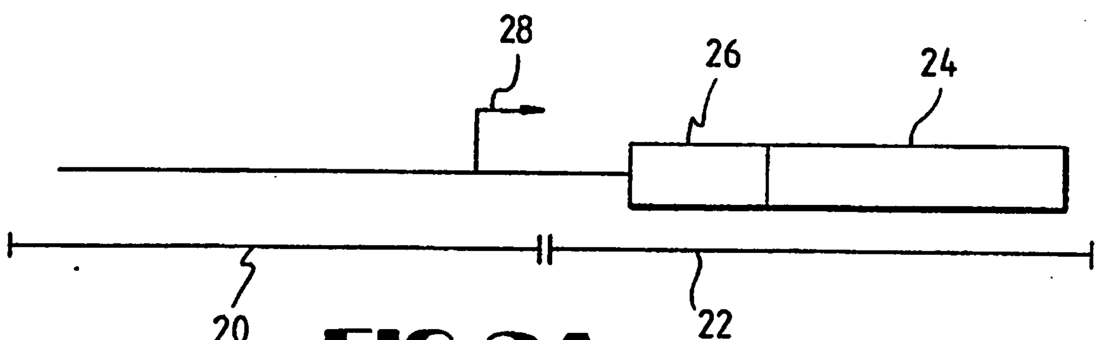


FIG.2A

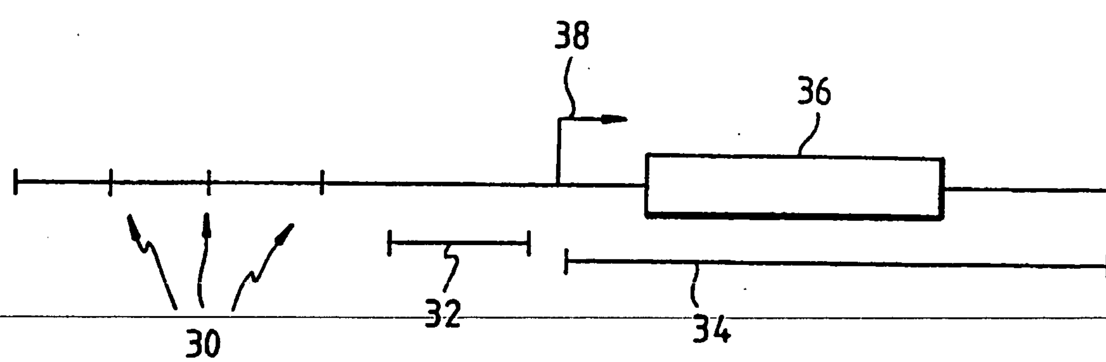


FIG.2B

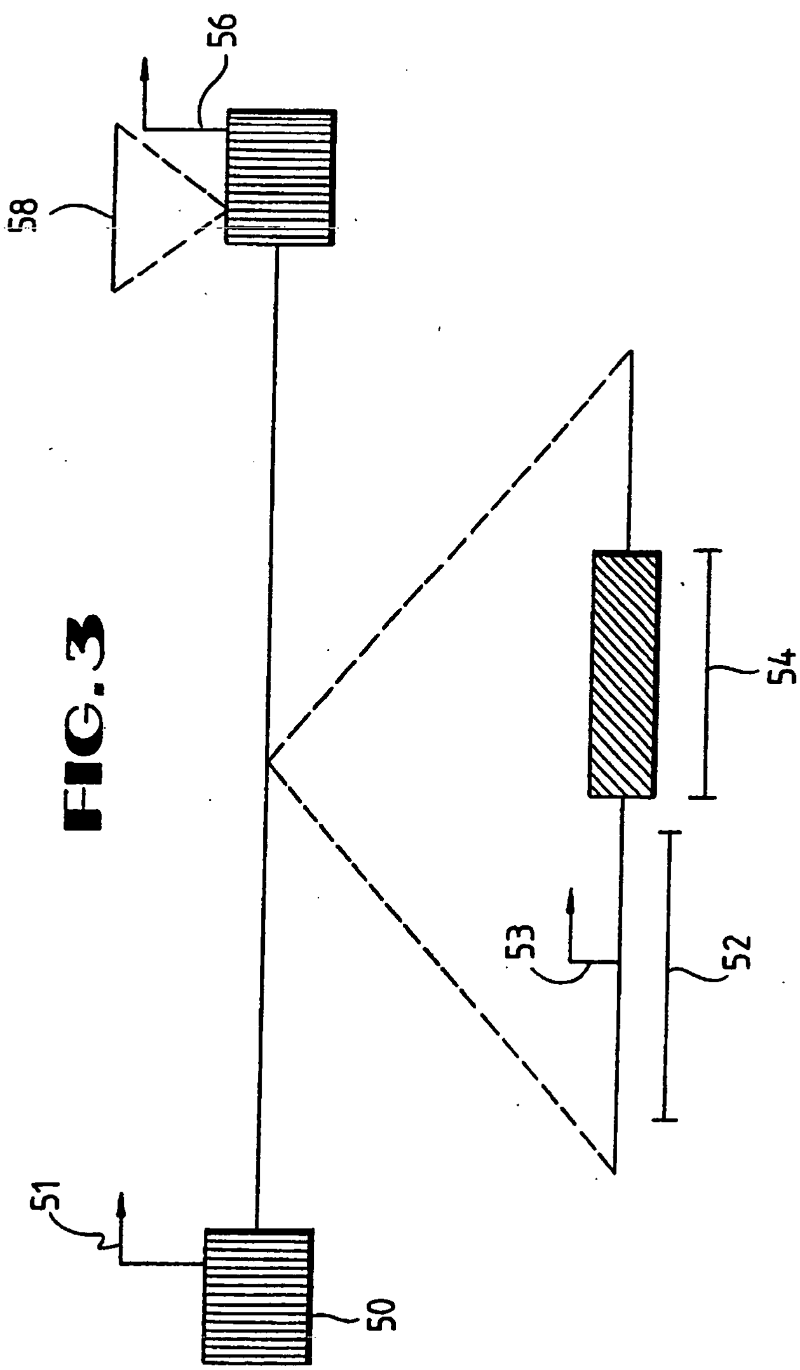


FIG. 3

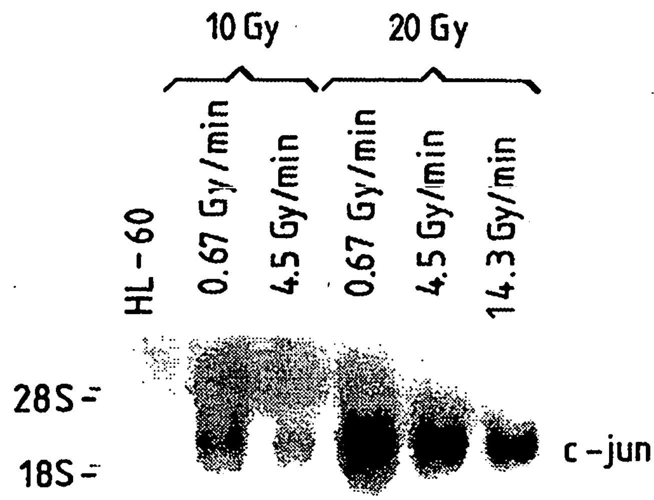


FIG.11

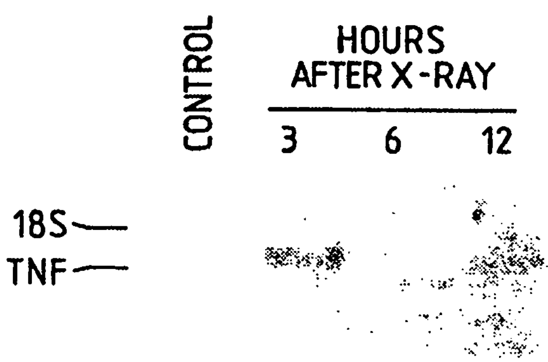


FIG.4A

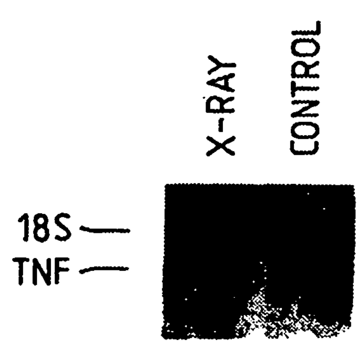


FIG.4B

FIG. 5A

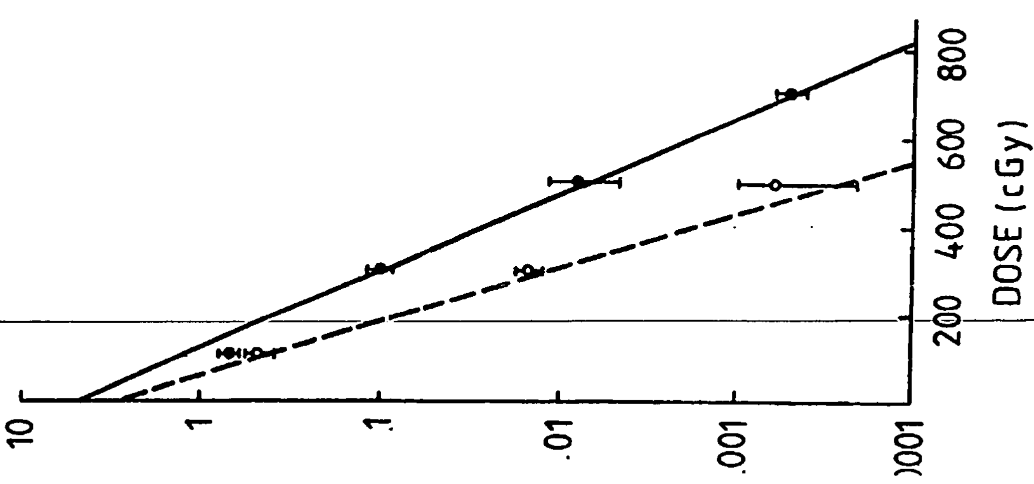


FIG. 5B

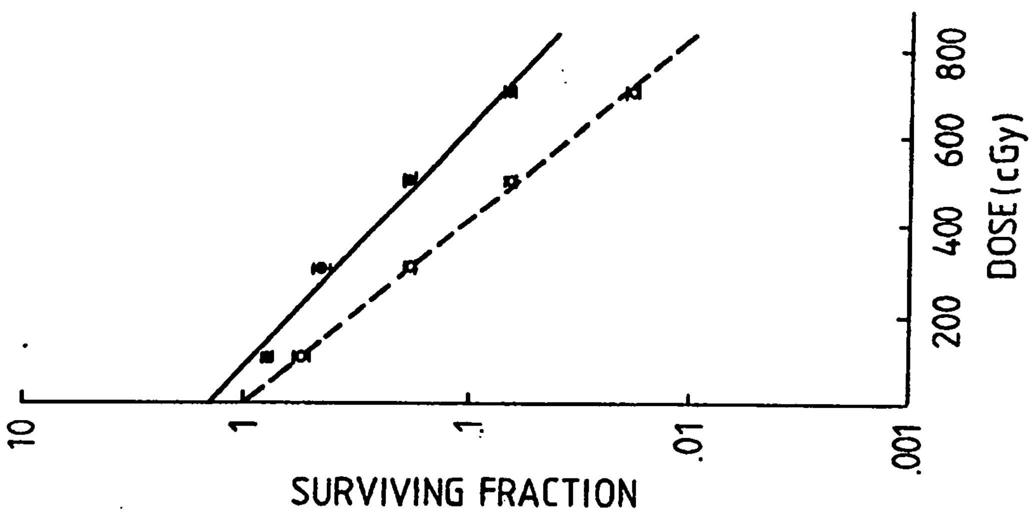
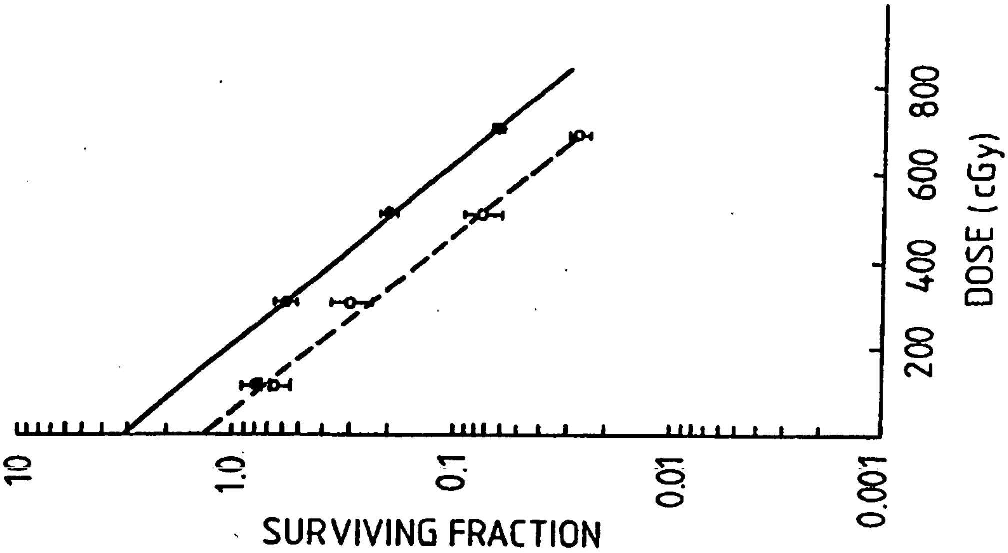


FIG. 5C



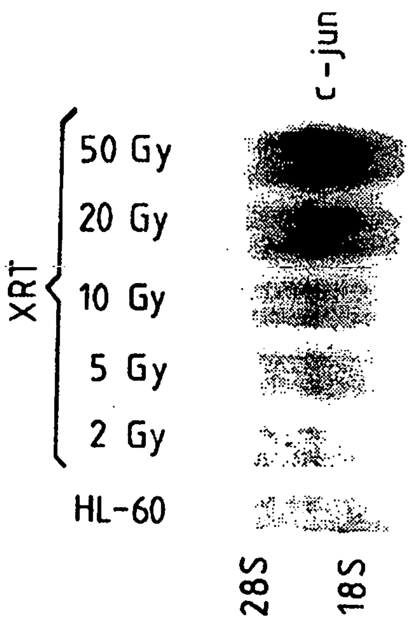


FIG.6B-1

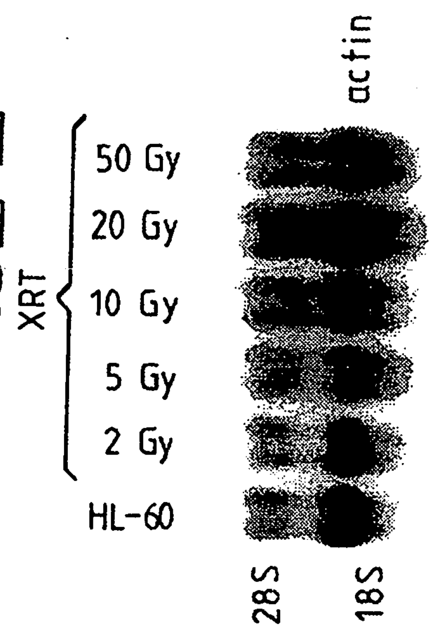


FIG.6B-2

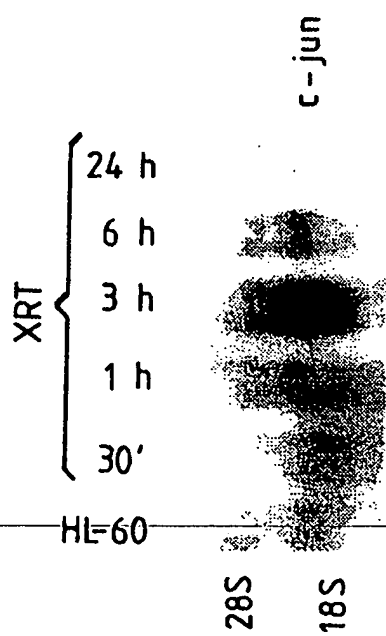


FIG.6A-1

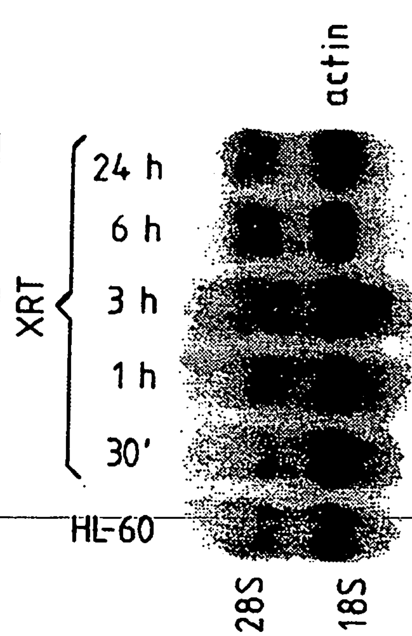


FIG.6A-2

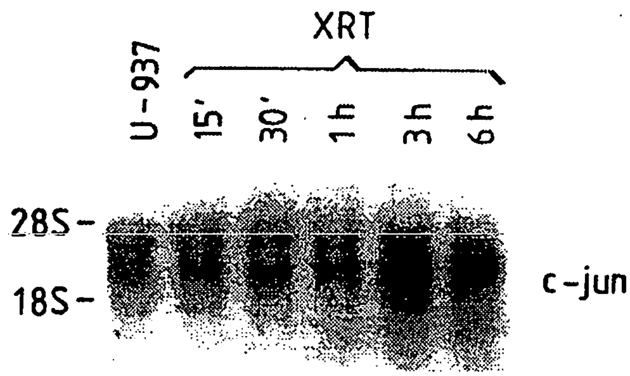

XRT
U-937
15'
30'
1h
3h
6h
28S
18S
c-jun

FIG.7A

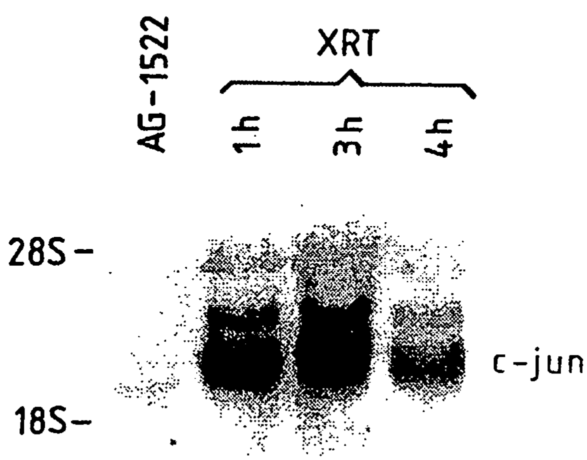

XRT
AG-1522
1h
3h
4h
28S
18S
c-jun

FIG.7B

FIG.8

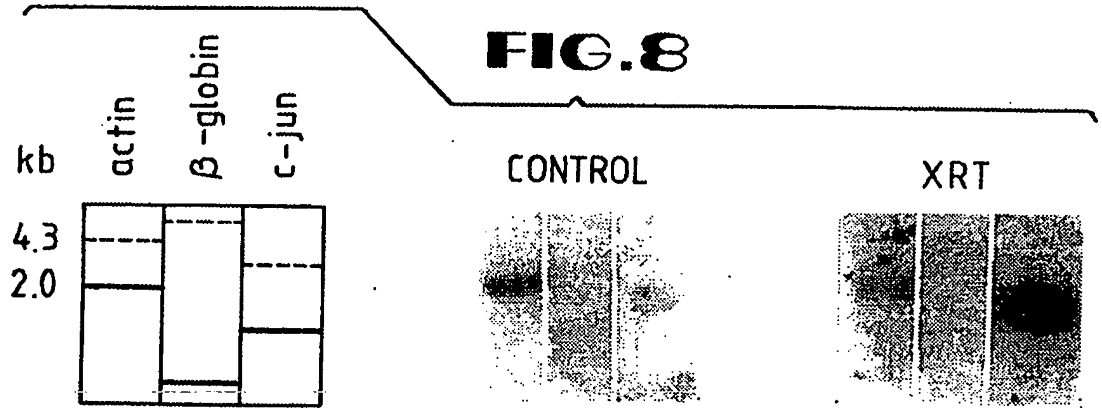
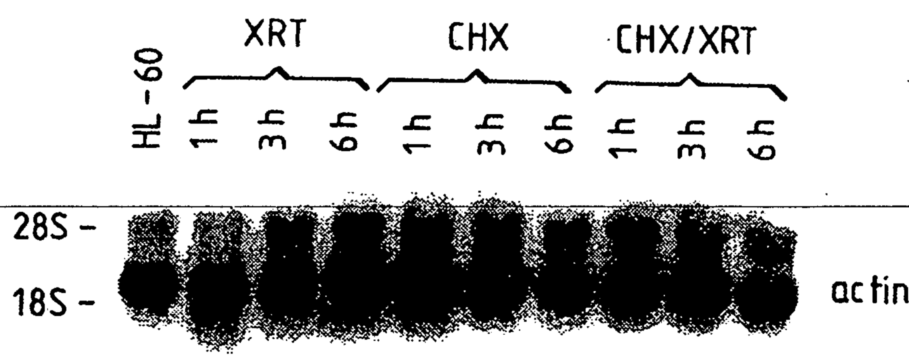


FIG.9A

FIG.9B



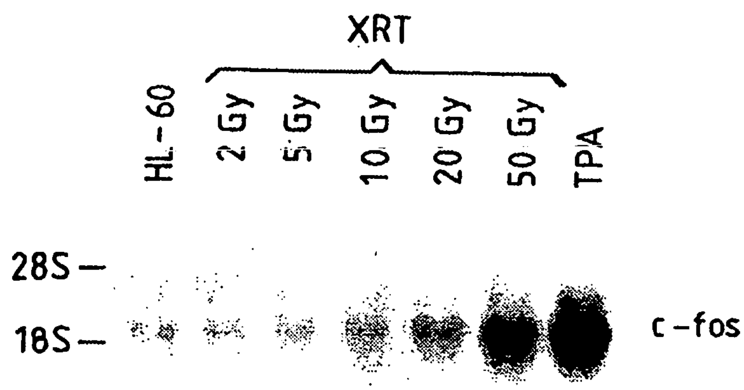


FIG.10A

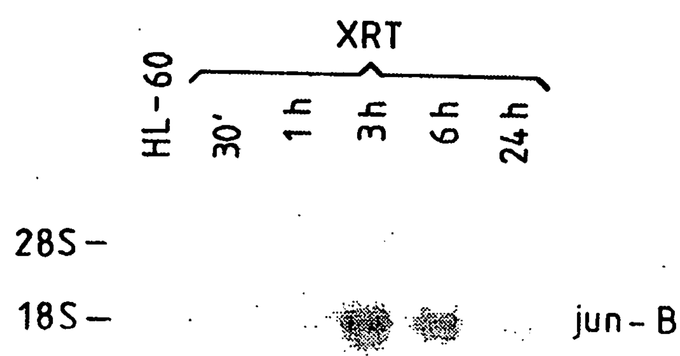


FIG.10B

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09651

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According t International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 43/00; C12Q 1/68; C07H 15/12; A01N 43/04
US CL : 424/1.1; 435/6; 536/27; 514/44; 935/6, 55, 62

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
US	424/1.1; 435/6; 536/27; 514/44; 935/6, 55, 62

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No. [18]
X	Int. J. Cancer, Volume 42, issued 1988, G. Sersa et al., "Anti-Tumor Effects of Tumor Necrosis Factor alone or combined with Radiotherapy", pages 129-134, see the entire document.	1-29
X	Cancer Research, Volume 49, Number 18, issued 15 September 1989, L. Witte, et al., "Effects of irradiation on the release of growth factors from cultured bovine, porcine, and human endothelial cells", pages 5066-5072, see abstract no. 07052277.	1-29
X	Proceedings of the National Academy of Science (USA), Volume 87, issued August 1990, M.L. Sherman, et al., "Ionizing radiation regulates expression of the c-jun protooncogene", pages 5663-5666, see entire document.	1-29
X	Proceedings of the National Academy of Science (USA), Volume 86, issued December 1989, D.E. Hallman, et al., "Increased tumor necrosis factor amRNA pages 10104-10107", see entire document.	1-29

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or mor ther such documents, such combination being obvious t a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Rep rt[2]
20 March 1992	02 APR 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	Bradl y Sisson